

-Supporting Information-

Neuroactive Chondroitin Sulfate Glycomimetics

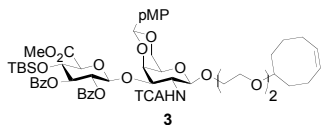
Manish Rawat, Cristal I. Gama, John B. Matson, and Linda C. Hsieh-Wilson*

*Division of Chemistry and Chemical Engineering and Howard Hughes Medical Institute, California
Institute of Technology, Pasadena, California 91125*

General Methods

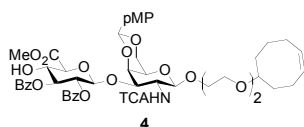
Unless otherwise stated, reactions were performed in flame-dried glassware under an argon atmosphere, using dry solvents. Solvents were dried by passage through an activated alumina column under argon. All other commercially obtained reagents were used as received unless otherwise noted. Thin layer chromatography (TLC) was performed using E. Merck silica gel 60 F254 precoated plates (0.25 mm). Visualization of the developed chromatogram was performed by UV, cerium ammonium molybdate and ninhydrin stain as necessary. ICN silica gel (particle size 0.032 - 0.063 mm) was used for flash chromatography. Gel filtration chromatography (Sephadex G-10 and G-25 ultrafine) was used in order to achieve purification of the final products.

¹H NMR and proton decoupling experiments were recorded on Varian Mercury 300 (300 MHz) and Varian Mercury 600 (600 MHz) spectrometers and are reported in parts per million (δ) relative to CDCl₃ (7.26 ppm), CD₃OD (4.87 ppm) and D₂O (4.80 ppm). Data for ¹H are reported as follows: chemical shift (δ ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet), coupling constant in Hz, and integration. ¹³C NMR spectra were obtained on a Varian Mercury 300 (75 MHz) spectrometer and are reported in terms of chemical shift. Mass spectra were obtained from the Protein/Peptide MicroAnalytical Laboratory and the Mass Spectrometry Facility at the California Institute of Technology.



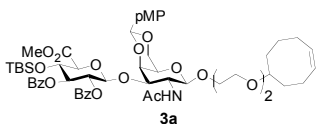
2-(2-(Cyclooct-4-enyloxy)ethoxy)ethyl (methyl 2,3-di-*O*-benzoyl-4-*O*-*tert*-butyldimethylsilyl- β -D-glucopyranosyluronate)-(1 \rightarrow 3)-4,6-*O*-*p*-methoxybenzylidene-2-deoxy-2-trichloroacetamido-D-galactopyranoside (3):

A mixture of donor **1** (0.750 g, 0.680 mmol) and acceptor **2** (0.300 g, 2.02 mmol) was co-evaporated with toluene (3 x 3 mL) and dried under vacuum overnight. The mixture was dissolved in CH₂Cl₂ (9.0 mL), and activated 4Å powdered molecular sieves were added. After stirring at rt for 30 min, the reaction was cooled to -75 °C and stirred for an additional 30 min. Trimethylsilyl trifluoromethanesulfonate (1 M in CH₂Cl₂, 125 μ L, 0.123 mmol) at -75 °C was added to the reaction dropwise. The reaction mixture was warmed to -20 °C, stirred for 30 min, quenched with triethylamine, filtered through Celite[®] and concentrated to afford a yellow syrup. Purification by flash chromatography (30% EtOAc:toluene) afforded **3** (0.460 g, 69%) as a white solid. R_f 0.40 (30% EtOAc:toluene). ¹H NMR (500 MHz, CDCl₃): δ 7.92 – 7.84 (m, 4 H, ArH), 7.54 – 7.46 (m, 2 H, ArH), 7.42 (d, *J* = 8.5 Hz, 2 H, C₆H₄OMe), 7.31 (dd, *J* = 7.5, 7.5 Hz, 2 H, ArH), 7.28 (dd, *J* = 7.5, 7.5 Hz, 2 H, ArH), 6.85 (d, *J* = 9.0 Hz, 2 H, C₆H₄OMe), 6.81 (d, *J* = 7.0 Hz, 1 H, NH), 5.68 – 5.51 (m, 2 H, CH=CH), 5.46 (dd, *J* = 8.5, 8.5 Hz, 1 H, H-3'), 5.43 (s, 1 H, MeOPhCH), 5.39 (dd, *J* = 7.0, 7.0 Hz, 1 H, H-2'), 5.06 (d, *J* = 7.5 Hz, 1 H, H-1'), 5.04 (d, *J* = 8.0 Hz, 1 H, H-1), 4.63 (dd, *J* = 3.5, 11.0 Hz, 1 H, H-3), 4.34 (d, *J* = 4.5 Hz, 1 H, H-4), 4.32 (dd, *J* = 8.5, 9.5 Hz, 1 H, H-4'), 4.26 (d, *J* = 12.0 Hz, 1 H, H-6), 4.09 (d, *J* = 9.5 Hz, 1 H, H-5'), 4.03 (d, *J* = 11.5 Hz, 1 H, H-7), 3.97 – 3.90 (m, 1 H, OCH₂CH₂O), 3.78 (s, 3 H, CO₂CH₃), 3.77 (s, 3 H, PhOCH₃), 3.76 – 3.62 (m, 2 H, H-2, OCH₂CH₂O), 3.60 – 3.54 (m, 2 H, OCH₂CH₂O), 3.53 – 3.39 (m, 5 H, H-5, OCH₂CH₂O), 3.32 – 3.26 (m, 1 H, OCH of COE), 2.36 – 2.22 (m, 1 H, CH₂ of COE), 2.18 – 1.84 (m, 3 H, CH₂ of COE), 1.93 – 1.82 (m, 1 H, CH₂ of COE), 1.80 – 1.58 (m, 3 H, CH₂ of COE), 1.48 – 1.28 (m, 2 H, CH₂ of COE), 0.70 (s, 9 H, SiC(CH₃)₃), -0.09 (s, 3 H, SiCH₃), -0.25 (s, 3 H, SiCH₃); ¹³C NMR (75 MHz, CDCl₃): δ 168.6, 165.5, 165.1, 162.1, 159.8, 133.2, 131.1, 130.3, 130.0, 129.8, 129.7, 129.4, 129.4, 129.0, 128.3, 128.3, 127.5, 113.4, 100.5, 100.2, 98.7, 81.0, 76.1, 75.4, 75.4, 73.4, 71.7, 70.8, 70.6, 70.4, 68.9, 68.4, 67.6, 67.6, 66.5, 55.3, 54.9, 52.5, 34.1, 34.1, 33.4, 33.4, 25.8, 25.6, 25.6, 25.4, 22.6, 17.7, -4.5, -5.2; ESI MS: *m/z* calcd for [C₅₅H₇₀C₁₃NO₁₇Si + H]⁺: 1150.3, obsd 1150.6.



2-(2-(Cyclooct-4-enyloxy)ethoxy)ethyl (methyl 2,3-di-*O*-benzoyl- β -D-glucopyranosyluronate)-(1 \rightarrow 3)-4,6-*O*-*p*-methoxybenzylidene-2-deoxy-2-

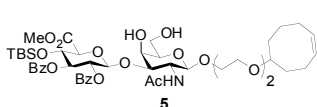
trichloroacetamido-D-galactopyranoside (4): A solution of **3** (0.410 g, 0.356 mmol) in dry THF (5.5 mL) and pyridine (5.5 mL) was cooled to 0 °C. HF•pyridine (1.30 mL, 70% HF in pyridine) was added, and the reaction mixture was warmed to rt and stirred for 12 h. The mixture was then diluted with EtOAc and washed with 10% aqueous CuSO₄. The aqueous phase was extracted with EtOAc (3x), and the combined organics were washed with saturated aqueous NaHCO₃ and dried over MgSO₄. The solvent was removed *in vacuo* to afford a yellow oil, which was purified by flash chromatography (50 \rightarrow 90% EtOAc:hexanes) to afford **4** (0.358 g, 95%) as a white solid. *R*_f 0.40 (80% EtOAc:hexanes). ¹H NMR (500 MHz, CDCl₃): δ 7.93 (dd, *J* = 1.0, 8.5 Hz, 2 H, Ar*H*), 7.89 (dd, *J* = 1.5, 8.0 Hz, 2 H, Ar*H*), 7.52 – 7.46 (m, 2 H, Ar*H*), 7.42 (d, *J* = 11.5 Hz, 2 H, C₆H₄OMe), 7.39 – 7.30 (m, 4 H, Ar*H*), 7.06 (d, *J* = 6.5 Hz, 1 H, *NH*), 6.87 (d, *J* = 11.5 Hz, 2 H, C₆H₄OMe), 5.68 – 5.54 (m, 2 H, CH=CH of COE), 5.48 (s, 1 H, MeOPhCH), 5.47 (dd, *J* = 8.0, 8.0 Hz, 1 H, H-2), 5.44 (dd, *J* = 8.0, 8.0 Hz, 1 H, H-3), 5.19 (d, *J* = 7.0 Hz, 1 H, H-1), 5.13 (d, *J* = 8.0 Hz, 1 H, H-1), 4.57 (ddd, *J* = 1.0, 3.5, 11.0 Hz, 1 H, H-3), 4.41 (d, *J* = 3.5 Hz, 1 H, H-4), 4.29 (dd, *J* = 1.5, 12.5 Hz, 1 H, H-6), 4.21 (dd, *J* = 9.0, 9.0 Hz, 1 H, H-4'), 4.10 (d, *J* = 10.0 Hz, 1 H, H-5'), 4.04 (dd, *J* = 1.5, 13.5 Hz, 1 H, H-7'), 3.97 (td, *J* = 5.0, 11.5 Hz, 1 H, OCH₂CH₂O), 3.85 (s, 3 H, CO₂CH₃), 3.81 (s, 3 H, PhOCH₃), 3.78 – 3.65 (m, 2 H, H-2, OCH₂CH₂O), 3.60 (t, *J* = 4.5 Hz, 2 H, OCH₂CH₂O), 3.55 – 3.42 (m, 6 H, H-5, OCH₂CH₂O, OH), 3.35 – 3.25 (m, 1 H, OCH of COE), 2.36 – 2.26 (m, 1 H, CH₂ of COE), 2.16 – 1.98 (m, 3 H, CH₂ of COE), 1.96 – 1.86 (m, 1 H, CH₂ of COE), 1.74 – 1.60 (m, 3 H, CH₂ of COE), 1.52 – 1.30 (m, 2 H, CH₂ of COE); ¹³C NMR (75 MHz, CDCl₃): δ 169.2, 166.5, 165.0, 162.1, 159.9, 133.4, 133.3, 130.2, 130.0, 129.9, 129.8, 129.4, 129.0, 128.8, 128.4, 128.4, 127.3, 113.4, 100.4, 100.3, 98.6, 92.2, 81.0, 77.2, 75.2, 74.0, 73.9, 71.1, 70.7, 70.4, 69.0, 68.5, 67.6, 66.5, 55.3, 55.0, 53.0, 34.1, 33.4, 25.7, 25.6, 22.6; ESI MS: *m/z* calcd for [C₄₉H₅₆NO₁₇Si + Na]⁺: 1058.2, obsd 1058.5.



2-(2-(Cyclooct-4-enyloxy)ethoxy)ethyl (methyl 2,3-di-*O*-benzoyl-4-*O*-tert-butyltrimethylsilyl- β -D-glucopyranosyluronate)-(1 \rightarrow 3)-4,6-*O*-*p*-

methoxybenzylidene-2-deoxy-2-acetamido- β -D-galactopyranoside (3a): Reduction of the *N*-

trichloroacetyl group was performed using a procedure modified from B  lot *et. al.*¹ To a solution of disaccharide **3** (0.250 g, 0.217 mmol) in benzene (7.8 mL) were added tributylstannane (305 μ L, 1.51 mmol) and 2,2'- azobisisobutyronitrile (0.080 g). After stirring at rt for 45 min, the reaction mixture was heated to 80 $^{\circ}$ C and stirred for an additional 1.5 h. The reaction was then cooled to rt and concentrated to afford a white solid. Purification by flash chromatography (50 \rightarrow 90% EtOAc:hexanes) afforded acetamide **3a** (0.209 g, 92%) as a white solid. R_f 0.30 (EtOAc). ¹H NMR (500 MHz, CDCl₃): δ 7.91 (d, *J* = 8.5 Hz, 2 H, Ar*H*), 7.90 (d, *J* = 8.5 Hz, 2 H, Ar*H*), 7.50 (dd, *J* = 1.0, 7.5 Hz, 2 H, Ar*H*), 7.46 (d, *J* = 8.5 Hz, 2 H, C₆H₄OMe), 7.40 – 7.32 (m, 4 H, Ar*H*), 6.89 (d, *J* = 10.0 Hz, 2 H, C₆H₄OMe), 5.70 – 5.52 (m, 3 H, H-3', CH=CH of COE), 5.47 (s, 1 H, MeOPhCH), 5.37 (dd, *J* = 6.5, 9.0 Hz, 1 H, H-2'), 5.08 (d, *J* = 8.5 Hz, 1 H, H-1), 5.00 (dd, *J* = 2.0, 7.5 Hz, 1 H, H-1'), 4.74 (td, *J* = 3.5, 7.5 Hz, 1 H, H-3), 4.37 (dd, *J* = 8.5, 10.0 Hz, 1 H, H-4'), 4.32 (d, *J* = 3.5 Hz, 1 H, H-4), 4.27 (dd, *J* = 1.0, 11.5 Hz, 1 H, H-6), 4.12 (d, *J* = 9.5 Hz, 1 H, H-5'), 4.05 (dd, *J* = 4.0, 12.0 Hz, 1 H, H-6), 3.95 (td, *J* = 4.5, 10.0 Hz, 1 H, OCH₂CH₂O), 3.82 (s, 3 H, CO₂CH₃), 3.79 (s, 3 H, PhOCH₃), 3.70 – 3.63 (m, 1 H, OCH₂CH₂O), 3.62 – 3.56 (m, 2 H, OCH₂CH₂O), 3.55 – 3.43 (m, 5 H, H-5, OCH₂CH₂O), 3.43 – 3.35 (m, 1 H, H-2), 3.35 – 3.28 (m, 1 H, OCH of COE), 2.38 – 2.28 (m, 1 H, CH₂ of COE), 2.18 – 2.08 (m, 1 H, CH₂ of COE), 2.08 – 1.98 (m, 1 H, CH₂ of COE), 1.96 – 1.84 (m, 1 H, CH₂ of COE), 1.82 – 1.60 (m, 4 H, CH₂ of COE), 1.55 (s, 3 H, NCOCH₃), 1.50 – 1.32 (m, 2 H, CH₂ of COE), 0.74 (s, 9 H, SiC(CH₃)₃), -0.05 (s, 3 H, SiCH₃), -0.21 (s, 3 H, SiCH₃); ¹³C NMR (75 MHz, CDCl₃): δ 171.3, 168.7, 165.6, 164.8, 159.8, 133.2, 133.1, 130.5, 129.7, 129.4, 129.3, 127.6, 113.3, 101.3, 100.5, 98.9, 81.0, 77.2, 76.0, 75.7, 75.5, 75.4, 72.1, 70.6, 70.5, 70.2, 69.1, 68.3, 67.5, 66.4, 55.2, 54.2, 52.5, 34.1, 33.4, 25.7, 25.6, 25.3, 23.2, 22.5, 17.7, -4.5, -5.2; ESI MS: *m/z* calcd for [C₅₅H₇₃NO₁₇Si + H]⁺: 1048.4, obsd 1048.5.



2-(2-(Cyclooct-4-enyloxy)ethoxy)ethyl (methyl 2,3-di-*O*-benzoyl-4-*O*-*tert*-butyldimethylsilyl- β -D-glucopyranosyluronate)-(1 \rightarrow 3)-2-deoxy-2-

acetamido- β -D-galactopyranoside (5): Acetamide **3a** (0.030 g, 0.029 mmol) was dissolved in CH₃CN (400 μ L) and H₂O (100 μ L). 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone (0.020 g, 0.089 mmol) was added. The reaction was stirred at rt for 2 h, quenched with MeOH and concentrated to yield a red solid. The product was purified on Sephadex LH-20 (50% CH₂Cl₂:MeOH) to afford the desired diol **5** as a white

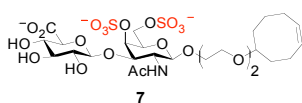
solid (0.024 g, 89%). R_f 0.12 (100% EtOAc). ¹H NMR (500 MHz, CDCl₃): δ 7.86 – 7.78 (m, 4 H, ArH), 7.52 – 7.44 (m, 2 H, ArH), 7.36 – 7.28 (m, 4 H, ArH), 5.67 – 5.47 (m, 3 H, H-3', CH=CH of COE), 5.31 (dd, *J* = 9.0, 10.0 Hz, 1 H, H-2'), 5.04 (dd, *J* = 1.5, 8.0 Hz, 1 H, H-1'), 4.39 (d, *J* = 8.5 Hz, 1 H, H-1), 4.26 (dd, *J* = 9.0, 9.0 Hz, 1 H, H-4'), 4.20 (dd, *J* = 2.0, 9.5 Hz, 1 H, H-5'), 4.03 (br s, 1 H, H-4), 3.94 – 3.85 (m, 1 H, H-2), 3.85 – 3.76 (m, 2 H, H-3, OCH₂CH₂O), 3.77 (s, 3 H, CO₂CH₃), 3.70 (dd, *J* = 6.0, 11.5 Hz, 1 H, H-6), 3.66 (dd, *J* = 5.5, 11.5 Hz, 1 H, H-6), 3.63 – 3.56 (m, 1 H, OCH₂CH₂O), 3.50 – 3.35 (m, 7 H, OCH₂CH₂O, H-5), 3.32 – 3.26 (m, 1 H, OCH of COE), 2.36 – 2.24 (m, 1 H, CH₂ of COE), 2.16 – 1.96 (m, 3 H, CH₂ of COE), 1.92 – 1.82 (m, 1 H, CH₂ of COE), 1.80 – 1.58 (m, 3 H, CH₂ of COE), 1.44 – 1.32 (m, 2 H, CH₂ of COE), 1.90 (d, *J* = 2.0 Hz, 3 H, NCOCH₃), 0.70 (s, 9 H, SiC(CH₃)₃), -0.05 (s, 3 H, CH₃Si), -0.22 (s, 3 H, CH₃Si); ¹³C NMR (75 MHz, CDCl₃): δ 170.4, 167.3, 166.6, 134.7, 134.6, 131.2, 131.1, 130.9, 130.9, 130.7, 130.7, 129.6, 103.7, 103.0, 82.9, 82.6, 77.4, 76.8, 76.3, 73.5, 72.3, 72.0, 71.7, 69.7, 69.4, 69.0, 68.9, 62.5, 53.4, 52.5, 35.5, 34.7, 34.6, 26.8, 26.7, 26.2, 23.7, 22.7, 18.8, -3.9, -4.6; ESI MS: *m/z* calcd for [C₄₇H₆₇NO₁₆Si + H]⁺: 930.4, obsd 930.7.



2-(2-(Cyclooct-4-enyloxy)ethoxy)ethyl (methyl 2,3-di-*O*-benzoyl-4-*O*-*tert*-butyldimethylsilyl-β-D-glucopyranosyluronate)-(1 → 3)-4,6-di-*O*-

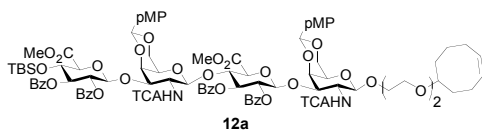
sodium sulfonato-2-deoxy-2-acetamido-β-D-galactopyranoside (6): Diol **5** (0.145 g, 0.156 mmol) was dissolved in DMF (7 mL), and SO₃•TMA (0.550 g, 3.96 mmol) was added. After stirring at 50 °C overnight, the reaction mixture was cooled to rt, quenched with MeOH and concentrated to afford a yellow solid. Purification on Sephadex LH-20 (50% CH₂Cl₂:MeOH) followed by silica gel chromatography (5:2:1 MeOH:EtOAc:H₂O) afforded **6** (0.144 g, 85%) as a white solid. R_f 0.35 (5:2:1 MeOH:EtOAc:H₂O). ¹H NMR (500 MHz, CD₃OD): δ 7.80 (d, *J* = 8.0 Hz, 2 H, ArH), 7.78 (d, *J* = 8.0 Hz, 2H, ArH), 7.45 (t, *J* = 7.0 Hz, 2 H, ArH), 7.34 – 7.26 (m, 4 H, ArH), 5.78 – 5.50 (m, 3 H, H-3', CH=CH of COE), 5.34 (dd, *J* = 7.5, 9.0 Hz, 1 H, H-2'), 5.05 (dd, *J* = 2.5, 7.0 Hz, 1 H, H-1'), 4.89 (s, 1 H, H-4), 4.40 (dd, *J* = 2.5, 8.0 Hz, 1 H, H-1), 4.35 (dd, *J* = 9.0 Hz, 1 H, H-4'), 4.33 (dd, *J* = 5.0, 11.5 Hz, 1 H, H-6), 4.17 (dd, *J* = 8.0, 11.0 Hz 1 H, H-6), 4.13 (d, *J* = 9.0 Hz, 1 H, H-5'), 4.00 – 3.92 (m, 1 H, H-2), 3.92 – 3.82 (m, 2 H, H-3, H-5), 3.82 – 3.74 (m, 1 H, OCH₂CH₂O), 3.76 (s, 3 H, CO₂CH₃), 3.64 – 3.56 (m, 1 H, OCH₂CH₂O), 3.54 – 3.34 (m, 6 H, OCH₂CH₂O), 3.34 – 3.26 (m, 1 H, OCH of COE), 2.34 – 2.24 (m, 1 H, CH₂ of COE), 2.12 – 2.04 (m, 2 H,

CH_2 of COE), 2.04 – 1.94 (m, 1 H, CH_2 of COE), 1.92 – 1.80 (m, 1 H, CH_2 of COE), 1.78 – 1.58 (m, 3 H, CH_2 of COE), 1.42 – 1.32 (m, 2 H, CH_2 of COE), 1.38 (s, 3 H, NHCOCH_3), 0.66 (s, 9 H, $\text{SiC}(\text{CH}_3)_3$), -0.07 (s, 3 H, SiCH_3), -0.23 (s, 3 H, SiCH_3); ^{13}C NMR (75 MHz, CDCl_3): δ 170.6, 170.3, 167.3, 167.0, 134.7, 134.6, 131.1, 130.9, 130.8, 130.8, 130.7, 130.6, 129.6, 129.5, 103.5, 103.1, 82.6, 82.6, 79.6, 77.4, 77.1, 74.2, 74.1, 72.0, 71.9, 71.6, 70.1, 69.1, 68.9, 68.9, 53.5, 52.7, 35.5, 35.4, 34.7, 34.6, 26.8, 26.7, 26.2, 23.7, 22.9, 18.8, -3.9, -4.5; ESI MS: m/z calcd for $[\text{C}_{47}\text{H}_{67}\text{NO}_{22}\text{S}_2\text{Si} + \text{Na} - 2 \text{H}]^-$: 1110.3, obsd 1110.5.



2-(2-(Cyclooct-4-enyloxy)ethoxy)ethyl (sodium β -D-glucopyranosyl-uronate)-(1 \rightarrow 3)-4,6-di-O-sodium sulfonato-2-deoxy-2-acetamido- β -D-

galactopyranoside (7): Disaccharide **6** (0.030 g, 0.027 mmol) was dissolved in pyridine (150 μL) and THF (150 μL) and cooled to 0 $^\circ\text{C}$. $\text{HF}\cdot\text{pyridine}$ (40 μL , 70% HF in pyridine) was added and the reaction was slowly warmed to rt. After 12 h, the mixture was passed through a Sephadex LH-20 column (50% CH_2Cl_2 :MeOH) to obtain the crude alcohol. The alcohol (0.025 g, 0.025 mmol) was dissolved in THF (400 μL) and H_2O (200 μL), and to this was added 1 M LiOH (100 μL) and H_2O_2 (50 μL , 30 wt% solution in H_2O). After stirring at rt for 12 h, 4 M NaOH (80 μL) and MeOH (400 μL) were added, and stirring was continued for another 12 h. The reaction was neutralized with Amberlyst IR-120 resin, filtered and lyophilized to afford an orange solid. The product was purified on Sephadex G-10 (100% H_2O) and Sephadex SP C25 (100% H_2O) and lyophilized to afford **7** (0.018 g, 89%, 2 steps) as a white solid. ^1H NMR (600 MHz, CD_3OD): δ 5.62 – 5.47 (m, 2 H, $\text{CH}=\text{CH}$ of COE), 4.68 (br s, 1 H, H-4), 4.44 (d, J = 7.8 Hz, 1 H, H-1), 4.33 (d, J = 7.8 Hz, 1 H, H-1'), 4.15 (d, J = 9.0 Hz, 1 H, H-6), 4.05 (dd, J = 9.6 Hz, 1 H, H-6'), 3.98 – 3.89 (m, 3 H, H-2, H-3, H-5), 3.88 – 3.82 (m, 1 H, $\text{OCH}_2\text{CH}_2\text{O}$), 3.72 – 3.64 (m, 1 H, $\text{OCH}_2\text{CH}_2\text{O}$), 3.60 – 3.46 (m, 6 H, H-5', $\text{OCH}_2\text{CH}_2\text{O}$), 3.46 – 3.43 (m, 1 H, $\text{OCH}_2\text{CH}_2\text{O}$), 3.39 (dd, J = 9.0, 9.0 Hz, 1 H, H-4'), 3.42 – 3.34 (m, 1 H, OCH of COE), 3.31 (dd, J = 9.0, 9.0 Hz, 1 H, H-3'), 3.22 (dd, J = 8.4, 8.4 Hz, 1 H, H-2'), 2.24 – 2.16 (m, 1 H, CH_2 of COE), 2.11 – 1.90 (m, 3 H, CH_2 of COE), 1.89 (d, J = 3.0 Hz, 3 H, CH_2 of COE), 1.88 – 1.80 (m, 1 H, CH_2 of COE), 1.68 – 1.48 (m, 3 H, NCOCH_3), 1.36 – 1.24 (m, 2 H, CH_2 of COE); ^{13}C NMR (75 MHz, CDCl_3): δ 178.4, 177.4, 132.8, 106.0, 103.7, 84.2, 79.0, 78.9, 77.8, 77.5, 75.4, 75.2, 75.0, 74.5, 72.7, 72.2, 71.8, 70.6, 69.4, 54.3, 36.1, 35.6, 27.9, 27.7, 25.0, 27.7; ESI MS: m/z calcd for $[\text{C}_{26}\text{H}_{43}\text{NO}_{20}\text{S}_2 + 2\text{Na} - 3\text{H}]^-$: 796.1, obsd 796.2.

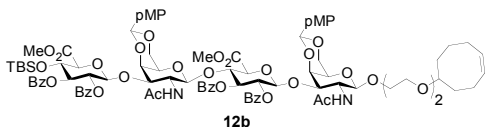


2-(2-(Cyclooct-4-enyloxy)ethoxy)ethyl (methyl 2,3-di-O-benzoyl-4-O-*tert*-butyldimethylsilyl- β -D-glucopyranosyl-

uronate)-(1 \rightarrow 3)-(4,6-O-*p*-methoxybenzylidene-2-deoxy-2-trichloroacetamido- β -D-galactopyranosyl)-(1 \rightarrow 4)-(methyl 2,3-di-O-benzoyl- β -D-glucopyranosyluronate)-(1 \rightarrow 3)-4,6-O-*p*-methoxybenzylidene-

2-deoxy-2-trichloroacetamido- β -D-galactopyranoside (12a): A mixture of donor **1** (0.050 g, 0.048 mmol) and acceptor **4** (0.064 g, 0.058 mmol) was co-evaporated with toluene (3 x 3 mL) and dried under vacuum overnight. The mixture was dissolved in CH₂Cl₂ (1.2 mL), and activated 4Å powdered molecular sieves were added. The reaction was cooled to -75 °C and stirred for 30 min. Trimethylsilyl trifluoromethanesulfonate (1 M in CH₂Cl₂, 11 μ L, 0.011 mmol) at -75 °C was added to the reaction dropwise. The reaction was warmed to -15 °C, stirred for 30 min, quenched with triethylamine, filtered and concentrated to afford a yellow syrup. Purification by flash chromatography (30% EtOAc:toluene) afforded the desired tetrasaccharide **12a** (0.044 g, 45%) as a white solid. R_f 0.50 (60% EtOAc:hexanes). ¹H NMR (500 MHz, CDCl₃): δ 7.92 – 7.88 (m, 4 H, ArH), 7.86 (dd, *J* = 1.0, 8.0 Hz, 2 H, ArH), 7.83 (dd, *J* = 1.0, 8.0 Hz, 2 H, ArH), 7.54 – 7.44 (m, 6 H, ArH, C₆H₄OMe), 7.37 – 7.26 (m, 8 H, ArH), 7.07 (d, *J* = 11.5 Hz, 2 H, C₆H₄OMe), 6.94 (d, *J* = 7.0 Hz, 2 H, C₆H₄OMe), 6.91 (d, *J* = 6.5 Hz, 1 H, NH), 6.74 (d, *J* = 6.5 Hz, 2 H, C₆H₄OMe), 6.66 (d, *J* = 8.5 Hz, 1 H, NH), 5.59 (dd, *J* = 8.0, 8.0 Hz, 1 H, H-3'), 5.70 – 5.55 (m, 2 H, CH=CH), 5.50 (s, 1 H, MeOPhCH), 5.45 (dd, *J* = 8.5, 8.5 Hz, 1 H, H-3'), 5.37 (dd, *J* = 7.0, 8.5 Hz, 2 H, H-2'), 5.20 (s, 1 H, MeOPhCH), 5.16 (d, *J* = 6.5 Hz, 1 H, H-1'), 5.11 (d, *J* = 8.0 Hz, 1 H, H-1), 5.04 (d, *J* = 7.0 Hz, 1 H, H-1'), 4.97 (d, *J* = 8.5 Hz, 1 H, H-1), 4.66 (ddd, *J* = 1.5, 3.5, 11.0 Hz, 1 H, H-3), 4.60 (dd, *J* = 9.5, 9.5 Hz, 1 H, H-4'), 4.42 – 4.32 (m, 3 H, H-3, H-4, H-4'), 4.30 (d, *J* = 12.5 Hz, 1 H, H-6), 4.17 (d, *J* = 9.5 Hz, 1 H, H-5'), 4.17 (s, 1 H, H-4), 4.09 (d, *J* = 9.5 Hz, 1 H, H-5'), 4.05 (d, *J* = 11.0 Hz, 1 H, H-6), 4.02 – 3.96 (m, 1 H, OCH₂CH₂O), 3.84 (s, 3 H, OCH₃), 3.81 (s, 6 H, OCH₃), 3.79 (s, 3 H, OCH₃), 3.86 – 3.66 (m, 5 H, H-2, H-6, OCH₂CH₂O), 3.64 – 3.60 (t, *J* = 4.5 Hz, 2 H, OCH₂CH₂O), 3.56 – 3.44 (m, 5 H, H-5, OCH₂CH₂O), 3.36 – 3.30 (m, 1 H, OCH of COE), 3.15 (br s, 1 H, H-5), 2.40 – 2.26 (m, 1 H, CH₂ of COE), 2.18 – 1.84 (m, 3 H, CH₂ of COE), 1.82 – 1.60 (m, 3 H, CH₂ of COE), 1.52 – 1.24 (m, 3 H, CH₂ of COE), 0.73 (s, 9 H, SiC(CH₃)₃), -0.80 (s, 3 H, SiCH₃), -0.23 (s, 3 H, SiCH₃); ¹³C NMR (125 MHz, CDCl₃): δ 168.6, 168.3, 165.5, 165.2, 165.1, 164.9, 162.1, 161.7, 159.9, 159.6, 133.2, 133.1, 132.9, 130.3, 130.0,

129.9, 129.8, 129.7, 129.6, 129.4, 129.3, 129.3, 129.0, 128.9, 128.3, 128.3, 128.2, 128.2, 127.0, 127.0, 113.4, 113.1, 100.5, 100.2, 100.1, 99.9, 98.7, 98.4, 80.9, 76.1, 75.5, 75.3, 74.8, 74.1, 73.7, 73.2, 73.1, 71.8, 71.6, 70.7, 70.5, 70.3, 69.0, 68.5, 68.1, 67.6, 67.6, 66.5, 55.3, 55.2, 55.0, 54.4, 53.1, 52.5, 34.1, 34.1, 33.4, 33.4, 25.7, 25.6, 25.6, 25.3, 22.6, 17.6, -4.5, -5.2; ESI MS: m/z calcd for $[C_{92}H_{104}Cl_6N_2O_{31}Si + H]^+$: 1971.4, obsd 1971.6.

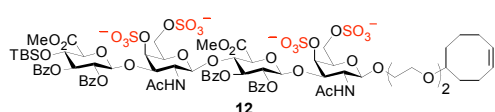


2-(2-(Cyclooct-4-enyloxy)ethoxy)ethyl (methyl 2,3-di-*O*-benzoyl-4-*O*-*tert*-butyldimethylsilyl- β -D-glucopyranosyl-

uronate)-(1 \rightarrow 3)-(4,6-*O*-*p*-methoxybenzylidene-2-deoxy-2-acetamido- β -D-galactopyranosyl)-(1 \rightarrow 4)-(methyl 2,3-di-*O*-benzoyl- β -D-glucopyranosyluronate)-(1 \rightarrow 3)-4,6-*O*-*p*-methoxybenzylidene-2-deoxy-

2-acetamido- β -D-galactopyranoside (12b): To a solution of tetrasaccharide **12a** (0.11 g, 0.056 mmol) in benzene (2.0 mL) were added tributylstannane (200 μ L, 0.74 mmol) and 2,2'-azobisisobutyronitrile (6.0 mg). After stirring at rt for 45 min, the reaction was then heated to 80 $^{\circ}$ C and stirred for an additional 3 h. The mixture was cooled to rt and concentrated to afford a white solid. Purification by flash chromatography (50 \rightarrow 100% EtOAc:hexanes) afforded the desired acetamide **12b** (0.088 g, 89 %) as a white solid. R_f 0.20 (EtOAc). 1H NMR (300 MHz, $CDCl_3$): δ 7.94 (d, J = 8 Hz, 2 H, ArH), 7.90 (d, J = 7.0 Hz, 2 H, ArH), 7.86 – 7.80 (m, 4 H, ArH), 7.50 – 7.40 (m, 6 H, ArH), 7.38 – 7.26 (m, 8 H, ArH), 7.19 (d, J = 7.5 Hz, 2 H), 6.83 (d, J = 8.5 Hz, 2 H, ArH), 6.78 (d, J = 7.0 Hz, 2 H), 5.68 (dd, J = 7.0, 12.0 Hz, 1 H), 5.68 – 5.50 (m, 4 H), 5.47 (dd, J = 8.5, 8.5 Hz, 1 H), 5.30 – 5.23 (m, 2 H, CH=CH of COE), 5.15 (s, 1 H), 5.01 (d, J = 8.5 Hz, 1 H), 4.98 (dd, J = 6.0, 12.0 Hz, 1 H), 4.96 – 4.90 (m, 1 H), 4.86 (d, J = 6.0 Hz, 1 H), 4.74 (dd, J = 6.0, 6.0 Hz, 1 H), 4.68 – 4.60 (m, 1 H), 4.53 (dt, J = 2.5, 9.1 Hz, 1 H), 4.38 (br s, 1 H), 4.26 (dd, 1 H, J = 9.0, 9.0 Hz), 4.24 (d, J = 9.0 Hz, 1 H), 4.16 (dd, J = 1.5, 9.5 Hz, 1 H, H-5'), 4.20 – 4.16 (m, 1 H), 4.03 (d, J = 10.0 Hz, 1 H, H-5'), 4.01 (d, J = 9.5 Hz, 1 H, H-6), 3.98 (d, J = 3.0 Hz, 1 H), 3.95 – 3.88 (m, 1 H, OCH_2CH_2O), 3.78 (s, 3 H, OCH_3), 3.80 – 3.76 (m, 1 H, H-6), 3.77 (s, 3 H, OCH_3), 3.71 (s, 3 H, OCH_3), 3.68 (s, 3 H, OCH_3), 3.66 – 3.60 (m, 1 H, OCH_2CH_2O), 3.58 – 3.54 (m, 3 H, OCH_2CH_2O), 3.50 – 3.45 (m, 3 H, OCH_2CH_2O , H-6), 3.45 – 3.37 (m, 3 H, H-2, H-5, OCH_2CH_2O), 3.37 – 3.26 (m, 2 H, OCH of COE, H-2), 2.77 (s, 1 H, H-5), 2.32 – 2.20 (m, 1 H, CH_2 of COE), 2.10 – 2.02 (m, 2 H, CH_2 of COE), 2.02 – 1.92 (m, 1 H, CH_2 of COE), 1.92 – 1.80 (m, 1 H, CH_2 of COE), 1.78 – 1.70 (m, 1 H, CH_2 of COE), 1.68 – 1.58 (m, 2

H, CH_2 of COE), 1.55 (s, 3 H, CH_3 of NCOCH_3), 1.47 (s, 3 H, NCOCH_3), 1.42 – 1.36 (m, 1 H, CH_2 of COE), 1.36 – 1.26 (m, 1 H, CH_2 of COE), 0.68 (s, 9 H, $\text{Si}(\text{CH}_3)_3$), -0.13 (s, 3 H, SiCH_3), -0.28 (s, 3 H, SiCH_3); ^{13}C NMR (125 MHz, CDCl_3): δ 171.3, 170.9, 168.6, 168.4, 165.6, 164.9, 164.8, 164.7, 159.7, 159.6, 133.2, 133.1, 130.7, 130.4, 129.9, 129.9, 129.8, 129.6, 129.5, 129.4, 129.4, 129.2, 129.1, 128.4, 128.4, 128.3, 127.7, 127.6, 113.4, 113.1, 101.0, 100.6, 100.2, 99.3, 99.1, 81.0, 77.2, 75.9, 75.7, 75.5, 75.3, 75.2, 75.1, 73.7, 73.6, 72.1, 72.0, 70.6, 70.5, 70.3, 69.2, 68.4, 68.2, 67.5, 66.4, 66.2, 55.3, 55.2, 54.1, 53.2, 52.7, 52.4, 34.1, 34.0, 33.5, 33.4, 25.7, 25.6, 25.3, 23.1, 23.1, 22.5, 17.7, -4.5, -5.2; ESI MS: m/z calcd for $[\text{C}_{92}\text{H}_{110}\text{N}_2\text{O}_{31}\text{Si} + \text{H}]^+$: 1767.6, obsd 1768.1.



2-(2-(Cyclooct-4-enyloxy)ethoxy)ethyl (methyl 2,3-di-*O*-benzoyl-4-*O*-*tert*-butyldimethylsilyl- β -D-glucopyranosyl-

uronate)-(1 \rightarrow 3)-(4,6-di-*O*-sodium sulfonato-2-deoxy-2-acetamido- β -D-galactopyranosyl)-(1 \rightarrow 4)-

(methyl 2,3-di-*O*-benzoyl- β -D-glucopyranosyluronate)-(1 \rightarrow 3)-4,6-di-*O*-sodium sulfonato-2-deoxy-2-

acetamido- β -D-galactopyranoside (12): Acetamide **12b** (0.038 g, 0.021 mmol) was dissolved in CH_3CN

(400 μL) and H_2O (40 μL). 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone (0.014 g, 0.063 mmol) was added.

The reaction was stirred at rt for 2 h, quenched with MeOH, and concentrated to yield a red solid. The

product was purified on Sephadex LH-20 (50% CH_2Cl_2 :MeOH) to afford the crude tetraol **12c** as a white

solid. The tetraol (0.026 g, 0.017 mmol) was dissolved in DMF (700 μL), and $\text{SO}_3 \cdot \text{TMA}$ (0.024 g, 3.96

mmol) was added. After stirring at 50 $^\circ\text{C}$ overnight, the reaction was cooled to rt, quenched with MeOH

and concentrated to afford a yellow solid. The product was purified on Sephadex LH-20 (50%

CH_2Cl_2 :MeOH) followed by silica gel chromatography (5:2:1 MeOH:EtOAc: H_2O) to afford **12** (0.022 g,

71%) as a white solid. R_f 0.45 (4:1:1 MeOH:EtOAc: H_2O). ^1H NMR (300 MHz, CD_3OD): δ 7.99 (d, J = 8.0

Hz, 2 H, ArH), 7.95 (dd, J = 1.0, 8.0 Hz, 2 H, ArH), 7.89 (dd, J = 1.0, 8.0 Hz, 2 H, ArH), 7.83 (dd, J = 1.0,

8.0 Hz, 2 H, ArH), 7.62 – 7.50 (m, 4 H, ArH), 7.44 (t, J = 7.0, 7.5 Hz, 4 H, ArH), 7.40 – 7.32 (m, 4 H,

ArH), 5.72 – 5.54 (m, 4 H, $\text{CH}=\text{CH}$, H-3'), 5.42 – 5.30 (m, 2H, H-2'), 5.14 (dd, J = 3.0, 6.0 Hz, 1 H), 5.05

(s, 1 H, H-4), 4.84 (d, J = 2.0 Hz, 1 H, H-4), 4.70 – 4.62 (m, 2 H), 4.48 (dd, J = 1.5, 8.0 Hz, 1 H), 4.46 –

4.40 (m, 2 H), 4.36 (dd, J = 1.5, 9.5 Hz, 1 H), 4.28 (dd, J = 7.5, 11.5 Hz, 1 H), 4.17 (d, J = 9.0 Hz, 1 H),

4.12 (dd, J = 5.5, 10.5 Hz, 1 H), 4.09 – 4.03 (m, 1 H), 3.98 – 3.80 (m, 7 H), 3.88 (s, 3 H, OCH_3), 3.83 (s, 3

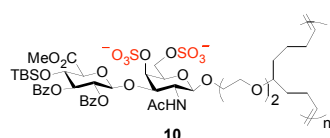
H, OCH_3), 3.76 – 3.66 (m, 2 H), 3.60 – 3.45 (m, 6 H), 3.42 – 3.36 (m, 1 H, OCH of COE), 2.42 – 2.30 (m, 1 H, CH_2 of COE), 2.20 – 2.12 (m, 2 H, CH_2 of COE), 2.10 – 2.00 (m, 1 H, CH_2 of COE), 2.00 – 1.90 (m, 1 H, CH_2 of COE), 1.80 – 1.60 (m, 3 H, CH_2 of COE), 1.51 (s, 3 H, NCOCH_3), 1.52 (s, 3 H, NCOCH_3), 1.54 – 1.36 (m, 2 H, CH_2 of COE), -0.75 (s, 9 H, $\text{SiC}(\text{CH}_3)_3$), -0.01 (s, 3 H, SiCH_3), -0.16 (s, 3 H, SiCH_3); ESI MS: m/z calcd for $[\text{C}_{76}\text{H}_{94}\text{N}_2\text{O}_{41}\text{S}_4\text{Si} + \text{Na} - 2\text{H}]^+$: 1871.4, obsd 1871.5.

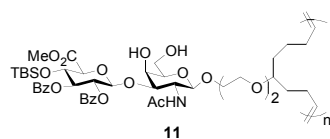
Polymerization Procedure for Unprotected CS Monomer 7: In a typical polymerization experiment, a small vial was charged with CS monomer **7** (15 mg, 0.20 mmol) and a small stir bar under the flow of argon. To this was added 200 μL of MeOH (for catalyst **8**) or H_2O (for the water-soluble catalyst, $\text{H}_2\text{IMes-poly(ethyleneglycol)(Cl)}_2\text{-Ru=CH}(o\text{-iPrOC}_6\text{H}_4)\text{)}$.³ The desired amount of bis-pyridine catalyst **8** (0.013 M stock solution in $(\text{CH}_2\text{Cl})_2$) or water-soluble catalyst (0.013 M stock solution in water) was added, and the reaction mixture was heated to 55 $^\circ\text{C}$. After stirring at 55 $^\circ\text{C}$ for 24 h, the reaction was quenched by the addition of ethylvinyl ether (150 μL), passed through a Sephadex G10 UF column (H_2O), and lyophilized to obtain **9a** or **9b** as an off-white solid. The lyophilized materials were characterized by ^1H NMR. The integrations of the phenyl peaks at 7.2 – 7.0 ppm and olefinic peaks at 5.4 – 5.2 ppm (cyclooctene) and 5.6 – 5.4 ppm (polymerized cyclooctene) were used to obtain the DP values and the extent of conversion. For the polymerization with **8** (2.5 mol%), 36% conversion was observed with a DP value of 21. For the polymerization with the water-soluble catalyst (2.5 and 5.0 mol%), incomplete conversion (7 and 60%, respectively) was observed with DP values of 8 and 28, respectively.

Polymerization Procedure for Protected CS Monomers: In a typical polymerization experiment, a small vial was charged with CS monomer (**5**, **6** or **12**; 0.018 mmol) and a small stir bar under the flow of argon. To this was added a solution of 5:1 $(\text{CH}_2\text{Cl})_2/\text{MeOH}$ (600 μL). The desired amount of bis-pyridine catalyst **8** (0.013 M stock solution in $(\text{CH}_2\text{Cl})_2$) was then added via syringe at rt. The reaction mixture was heated to 55 $^\circ\text{C}$ and stirred at this temperature for 24 h. At this point, TLC of the crude reaction mixture showed complete consumption of starting material. The polymerization process was quenched by the addition of ethyl vinyl ether (0.3 mL), and the resultant precipitate was filtered and washed with diethyl ether. The ^1H NMR of the crude product showed the disappearance of the cyclooctene methylene protons between 2.4-

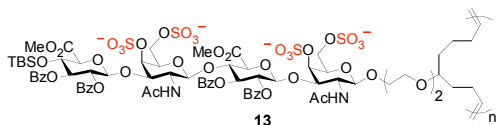
2.2 ppm, indicating the completion of the polymerization reaction. Further confirmation of the polymerization reaction was established by ^1H NMR of the crude product after desilylation and saponification, followed by salt removal using Sephadex G10 UF (H_2O). The ^1H NMR of the deprotected product showed a shift of the olefinic protons from 5.6 – 5.4 ppm (sharp multiplet corresponding to cyclooctene olefinic protons) to 5.4 – 5.2 ppm (broad peak corresponding to the poly(cyclooctene) backbone protons).

Small CS oligomers were removed from the glycopolymer by dialysis at rt against 0.2 M LiBr in DMSO (Spectra/Por Biotech Dialysis Membranes RC MWCO 8000, 25000 or 50000; 2 x 12 h). The polymer was then dialyzed at rt against DMSO without LiBr (3 x 6 h) and lyophilized to dryness to yield a white solid. All glycopolymers were characterized by ^1H NMR spectroscopy and gel permeation chromatography (GPC). GPC was carried out in 0.2 M LiBr in DMF on two I-series Mixed Bed Low MW ViscoGel columns (Viscotek) connected in series with a DAWN EOS multiangle laser light scattering (MALLS) detector and an Optilab DSP differential refractometer (both from Wyatt Technology). No calibration standards were used, and dn/dc values were obtained for each injection assuming 100% mass elution from the columns. The polymerization yield and M_w , M_n and PDI values for different CS monomers and different catalyst loadings are shown in Table 1.

 **CS-E disaccharide protected polymer (10):** 53 – 88% yield for 0.5 – 5.0 mol% catalyst. ^1H NMR (500 MHz, D_2O): δ 8.10 – 7.45 (m, 10 H), 5.60 – 5.02 (m, 6 H, H-1', H-2', H-3', $\text{CH}=\text{CH}$), 4.65 – 4.00 (m, 6 H, H-1, H-4, H-5, H-6, H-4'), 4.00 – 3.20 (m, 11 H, H-2, H-3, H-5, $\text{CH}_2\text{CH}_2\text{O}$), 3.76 (s, 3 H, COOCH_3), 3.20 – 3.10 (m, 1 H, OCH of COE), 2.04 – 1.80 (m, 3 H), 1.49 (s, 3 H), 1.43 – 1.11 (m, 7 H), 0.66 (s, 9 H, $\text{SiC}(\text{CH}_3)_3$), -0.06 (s, 3 H, SiCH_3), -0.24 (s, 3 H, SiCH_3).

 **Unsulfated CS disaccharide protected polymer (11):** 69% yield. ^1H NMR (500 MHz, D_2O): δ 7.97 (d, J = 7.5 Hz, 2 H, ArH), 7.88 (d, J = 7.5 Hz, 2 H, ArH), 7.72 (dd, J = 7.0, 7.0 Hz, 1 H, ArH), 7.67 (dd, J = 7.0, 7.0 Hz, 1 H, ArH), 7.59 (dd, J = 7.5, 7.5 Hz, 2 H, ArH), 7.53 (dd, J = 7.5, 7.5 Hz, 2 H, ArH), 5.75 (dd, J = 9.0, 9.0 Hz, 1 H, H-3'), 5.56 – 5.52

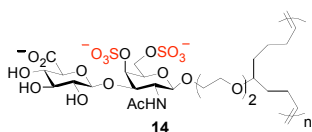
(m, 2 H, $CH=CH$), 5.38 (dd, $J = 8.0, 8.0$ Hz, 1 H, H-2'), 5.34 (d, $J = 7.5$ Hz, 1 H, H-1'), 4.56 – 4.50 (m, 2 H, H-5'), 4.46 – 4.40 (m, 1 H), 4.37 (dd, $J = 9.0, 9.0$ Hz, 1 H, H-4'), 4.06 – 4.00 (m, 1 H, H-4), 3.98 – 3.70 (m, 3 H, H-2, H-3, OCH_2CH_2O), 3.90 (s, 3 H, OCH_3) 3.72 – 3.40 (m, 9 H), 3.38 – 3.28 (m, 1 H, OCH of COE), 2.20 – 2.00 (m, 4 H), 1.62 – 1.40 (m, 6 H), 1.36 (s, 3 H, $NCOCH_3$), 0.84 (s, 9 H, $SiC(CH_3)_3$), 0.09 (s, 3 H, $SiCH_3$), -0.08 (s, 3 H, $SiCH_3$).



CS-E tetrasaccharide protected polymer (13): 51% yield.

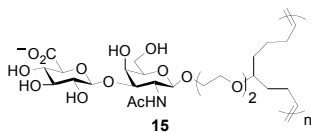
1H NMR (500 MHz, D_2O): δ 8.00 – 7.20 (m, 20 H), 5.60 – 4.92 (m, 9 H, H-1, H-1', H-2', H-3', $CH=CH$), 4.60 – 4.02 (m, 11 H, H-1, H-4, H-5, H-6, H-4'), 3.85 (s, 3 H, $COOCH_3$), 3.77 (s, 3 H, $COOCH_3$), 3.20 (m, 3.90, 14 H, H-2, H-3, H-5, OCH_2CH_2O), 3.20 – 3.10 (m, 1 H, OCH of COE), 2.03 – 1.80 (m, 3 H), 1.53 (s, 3 H), 1.42 (s, 3 H), 1.50 – 1.10 (m, 7 H), 0.66 (s, 9 H, $SiC(CH_3)_3$), -0.07 (s, 3 H, $SiCH_3$), -0.27 (s, 3 H, $SiCH_3$).

Polymer Deprotection Procedure: The glycopolymer (**10**, **11** or **13**; 0.018 mmol) was dissolved in pyridine (120 μ L), THF (120 μ L) and H_2O (28 μ L). The reaction was cooled to 0 $^{\circ}C$, and $HF \cdot \text{pyridine}$ (33 μ L, 70% HF in pyridine) was added. After stirring at 0 $^{\circ}C$ for 1 h and at rt overnight, the reaction was quenched with methoxytrimethylsilane to obtain a brown precipitate. The precipitate was filtered, washed with ether and subjected to saponification conditions. The alcohol was deprotected in a manner similar to a procedure from Lucas *et. al.*² To a solution of the alcohol in THF (152 μ L) and H_2O (75 μ L) at 0 $^{\circ}C$ were added 1 M aq. $LiOH$ (60 μ L) and 30% H_2O_2 (30 μ L). The reaction was stirred at 0 $^{\circ}C$ for 1 h and at rt for 12 h. At this time, 4 M $NaOH$ (45 μ L) and $MeOH$ (224 μ L) were added, and the reaction was stirred for another 12 h. It was then neutralized with Amberlyst IR-120 resin, filtered and lyophilized to afford an orange solid. The product was purified by Sephadex G-25 UF (0.9 % $NaCl$ in H_2O) and desalted with Sephadex G-25 UF (100% H_2O) to afford **14** (87 – 95% yield), **15** (69% yield) or **16** (87% yield) as a white solid upon lyophilization.



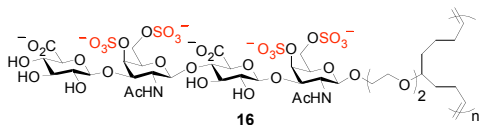
CS-E disaccharide polymer (14): 87 – 95% yield for 0.5 – 5.0 mol%

catalyst. ^1H NMR (500 MHz, D_2O): δ 5.46 – 5.30 (m, 2 H, $\text{CH}=\text{CH}$), 4.71 (s, 1 H, H-4), 4.46 (d, $J = 5.0$ Hz, 1 H, H-1), 4.37 (d, $J = 7.5$ Hz, 1 H, H-1'), 4.17 (d, $J = 10.5$ Hz, 1 H, H-6), 4.07 (dd, $J = 9.5, 9.5$ Hz, 1 H, H-6), 4.02 – 3.82 (m, 4 H, H-2, H-3, H-5, $\text{OCH}_2\text{CH}_2\text{O}$), 3.74 – 3.48 (m, 8 H, H-5', $\text{OCH}_2\text{CH}_2\text{O}$), 3.41 (dd, $J = 8.5$ Hz, 1 H, H-4'), 3.36 (dd, $J = 8.0, 8.5$ Hz, 1 H, H-3'), 3.40 – 3.30 (m, 1 H, OCH of COE), 3.23 (dd, $J = 8.0, 8.0$ Hz, 1 H, H-2'), 1.91 (s, 3 H, NCOCH_3), 2.08 – 1.80 (m, 4 H, CH_2 of COE), 1.70 – 1.10 (m, 6 H, CH_2 of COE).



Unsulfated CS disaccharide polymer (15): 69% yield. ^1H NMR (500

MHz, D_2O): δ 5.60 – 5.20 (m, 2 H, $\text{CH}=\text{CH}$), 4.34 (d, $J = 8.0$ Hz, 1 H, H-1), 4.30 (d, $J = 7.0$ Hz, 1 H, H-1'), 4.11 (d, $J = 2.0$ Hz, 1 H, H-4), 4.00 – 3.90 (m, 2 H, H-2, H-6), 3.80 – 3.50 (m, 13 H, H-3, H-5, H-6, H-5', OCH of COE, $\text{OCH}_2\text{CH}_2\text{O}$), 3.44 (dd, $J = 9.0, 9.0$ Hz, 1 H, H-3'), 3.41 (dd, $J = 9.5, 9.5$ Hz, 1 H, H-2'), 3.28 (dd, $J = 8.0, 8.0$ Hz, 1 H, H-1'), 1.96 (s, 3 H, NCOCH_3), 2.40 – 1.80 (m, 4 H), 1.70 – 1.00 (m, 6 H).



CS-E tetrasaccharide polymer (16): 87% yield. ^1H NMR

(500 MHz, D_2O): δ 5.60 – 5.30 (m, 2 H, $\text{CH}=\text{CH}$), 4.74 – 4.72 (m, 2 H, H-4), 4.62 – 4.52 (m, 2 H, H-1), 4.48 (d, $J = 7.0$ Hz, 1 H, H-1'), 4.46 (d, $J = 7.0$ Hz, 1 H, H-1'), 4.30 – 3.90 (m, 11 H, H-2, H-3, H-5, H-6, $\text{OCH}_2\text{CH}_2\text{O}$), 3.84 – 3.52 (m, 12 H, H-3', H-4', H-5', H-5', OCH of COE, $\text{OCH}_2\text{CH}_2\text{O}$), 3.49 (dd, $J = 8.5, 8.5$ Hz, 1 H, H-4'), 3.43 (dd, $J = 8.0, 8.0$ Hz, 1 H, H-3'), 3.36 (dd, $J = 8.5, 8.5$ Hz, 1 H, H-2'), 3.30 (dd, $J = 8.0, 8.0$ Hz, 1 H, H-2'), 1.94 (s, 3 H, NCOCH_3), 1.91 (s, 3 H, NCOCH_3), 2.20 – 1.60 (m, 4 H, CH_2 of COE), 1.60 – 1.10 (m, 6 H, CH_2 of COE).

Treatment of Hippocampal Neurons with the CS-E Polysaccharide and CS Glycopolymers.

Hippocampal neuronal cultures were prepared using a procedure modified from Goslin and Banker.⁴ Briefly, hippocampi of embryonic day 18 (E18) rats were dissected and transferred to 4.5 mL of ice-cold Calcium and Magnesium Free-Hank's Balanced Salt Solution (CMF-HBSS). Trypsin (2.5%, no EDTA) was added to 5 mL, and the tissue was incubated for 15 min at 37 °C. The trypsin solution was removed

and the tissue sample washed three times with 5 mL of CMF-HBSS. Cells were then dissociated from the tissue in 1 mL of CMF-HBSS by passing through a P1000 pipette tip twenty times. The cells were counted, diluted into Minimal Eagle's Medium (MEM) plus 10% fetal bovine serum, and seeded on glass coverslips (Carolina Biological) coated with 15 $\mu\text{g/mL}$ poly-DL-ornithine at a density of 70 cells/ mm^2 for 30 min. After this time, 500 μL of supplemented Neurobasal medium (47.5 mL Neurobasal medium without L-glutamine; 0.5 mL L-glutamine (200 mM); 0.5 mL penicillin/streptomycin (10,000 U/mL); 0.5 mL antibiotic-antimycotic (100X stock); 1.0 mL B-27 serum-free supplement (50X stock); 50 μL of 0.5 M kynurenic acid in 1 N NaOH) was added to each coverslip. The cultures were incubated in 5% CO_2 at 37 $^\circ\text{C}$ for 24 h. The media was then removed, and a 1.25 μL solution of CS-E polysaccharide (Seikagaku; ~60% of the polysaccharide is estimated to contain the CS-E motif) or glycopolymer in 3.5 M aq. NaCl was added to supplemented Neurobasal medium (498.75 μL) on each coverslip. A fixed uronic acid concentration of 0.5 $\mu\text{g/mL}$ was used in each case to compare the effects of multivalency. This concentration corresponded to molar concentrations of 14.3 nM for the natural CS-E polysaccharide and 53.0 nM, 33.2 nM, 22.9 nM, and 16.6 nM for glycopolymer **14** with DP values of 25, 40, 58 and 80, respectively. The molar concentrations of glycopolymers **11** and **16** were 43.2 nM and 12.1 nM, respectively. Importantly, no cellular toxicity was observed at the concentrations used for each compound, as demonstrated by adherence of the cells to the coverslip and healthy cellular morphology. The cultures were incubated for an additional 24 h in 5% CO_2 at 37 $^\circ\text{C}$ and analyzed as described below.

Concentrations of the CS-E polysaccharide and glycopolymers were determined by measuring their relative uronic acid contents using the carbazole reaction.⁵ Briefly, the acid borate reagent (1.5 mL of 0.80 g sodium tetraborate, 16.6 mL H_2O , and 83.3 mL H_2SO_4) was added to 15-mL glass test tubes. The polysaccharide and glycopolymers (5 μL of a 10 mg/mL stock in 3.5 M NaCl) were added and the solution placed in a boiling H_2O bath for 10 min. Following addition of the carbazole reagent (50 μL of 0.1% w/v carbazole in 100% EtOH), the solution was boiled for 15 min. The absorbance was read at 530 nm and compared to a D-glucuronolactone standard in H_2O . From these readings, stock solutions containing 200 $\mu\text{g/mL}$ uronic acid in 3.5 M aq. NaCl were made for each compound and used for treatments of neurons.

Determining the Relative Potencies of the Natural Polysaccharide and Glycopolymer 16.

Hippocampal neurons were grown for 24 h before medium was replaced with fresh supplemented Neurobasal medium, and compounds were added at various uronic acid concentrations ranging from 0.01 to 0.5 $\mu\text{g/mL}$. Neurons were incubated with the compounds for 24 h and then analyzed as described below. To determine the IC_{50} values, the concentrations of the CS-E polysaccharide were calculated based on an average molecular weight of 70,000 g/mol, as provided by the manufacturer. For glycopolymer **16**, a molecular weight of 84,096 g/mol, as determined by GPC, was used. The concentration values were plotted against the % inhibition of neurite outgrowth relative to untreated neurons, and the IC_{50} values represent molar concentrations of compound needed for 50% inhibition of neurite outgrowth. IC_{50} values of 1.2 ± 0.1 nM and 1.3 ± 0.1 nM were determined for the natural polysaccharide and glycopolymer **16**, respectively (1.2 ± 0.1 nM and 1.6 ± 0.1 nM if calculated based on the saccharide content of each molecule).

Immunocytochemistry of Hippocampal Neuronal Cultures. After 48 h in culture, the hippocampal neurons on coverslips were rinsed one time with PBS, fixed in 4% paraformaldehyde for 20 min at rt, washed twice with PBS, permeabilized in 0.3% Triton X-100 for 5 min at rt, and washed twice with PBS. Non-specific binding was blocked by incubating with 3% BSA for 1 h at rt and then rinsing once with PBS. Cells were then incubated with anti-tubulin antibodies (rabbit polyclonal, 1:500; Sigma) in 3% BSA overnight at 4 °C. Excess antibody was rinsed away 5 times with PBS. Secondary antibody, anti-rabbit IgG AlexaFluor 488 (1:500; Molecular Probes), was added for 1 h at 37 °C in 3% BSA. Excess secondary antibody was washed off 5 times with PBS. The coverslips were mounted onto glass slides using Vectashield mounting medium (Vector Labs) and sealed with clear nail polish. Cells were imaged on a Nikon Eclipse TE2000-S inverted microscope. The images were captured with MetaMorph 6.1 software using a 40x plan fluor oil objective.

Morphometric Analysis. All experiments were performed in triplicate. For each experiment, 25 randomly selected cells were analyzed per coverslip. To ensure accurate measurements, only neurites longer than ~ 10 μm and not in contact with other cells were measured using MetaMorph 6.1 software. The

mean neurite lengths were compared among the different conditions by the ANOVA test using the statistical analysis program in KaleidaGraph 4.0.

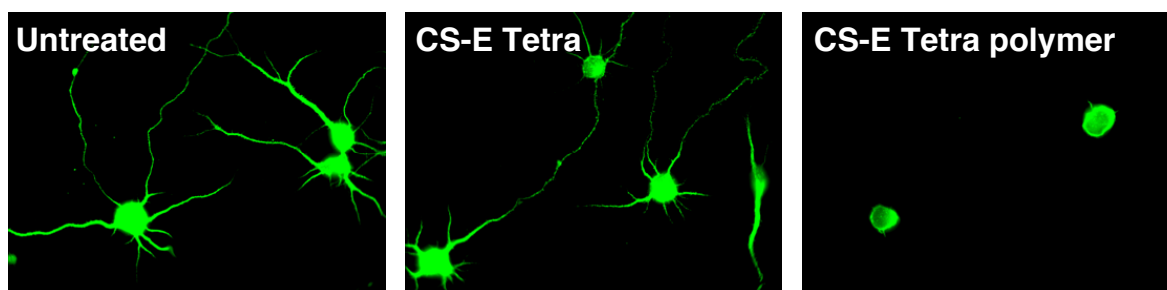


Figure S1. A monovalent CS-E tetrasaccharide displays no inhibitory activity, whereas the CS-E tetrasaccharide glycopolymer **16** completely inhibits neurite outgrowth. Hippocampal neurons were treated with 0.5 $\mu\text{g/mL}$ uronic acid equivalents of a monovalent tetrasaccharide⁶ or glycopolymer **16**.

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